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Advanced purification strategy for CueR, a cysteine containing copper(I) and DNA binding protein - DTU Orbit (09/11/2017)

Advanced purification strategy for CueR, a cysteine containing copper(I) and DNA binding protein

Metal ion regulation is essential for living organisms. In prokaryotes metal ion dependent transcriptional factors, the so-called metalloregulatory proteins play a fundamental role in controlling the concentration of metal ions. These proteins recognize metal ions with an outstanding selectivity. A detailed understanding of their function may be exploited in potential health, environmental and analytical applications. Members of the MerR protein family sense a broad range of mostly late transition and heavy metal ions through their cysteine thiolates. The air sensitivity of latter groups makes the expression and purification of such proteins challenging. Here we describe a method for the purification of the copper-regulatory CueR protein under optimized conditions. In order to avoid protein precipitation and/or eventual aggregation and to get rid of the co-purifying *Escherichia coli* elongation factor, our procedure consisted of four steps supplemented by DNA digestion. Subsequent anion exchange on Sepharose FF Q 16/10, affinity chromatography on Heparin FF 16/10, second anion exchange on Source 30 Q 16/13 and gel filtration on Superdex 75 26/60 resulted in large amounts of pure CueR protein without any affinity tag. Structure and functionality tests performed with mass spectrometry, circular dichroism spectroscopy and electrophoretic gel mobility shift assays approved the success of the purification procedure.

General information

State: Published
Organisations: Department of Chemistry, Metalloprotein Chemistry and Engineering, Organic Chemistry, University of Szeged, Hungarian Academy of Sciences
Authors: Balogh, R. K. (Ekstern), Gyurcsik, B. (Ekstern), Hunyadi-Gulyás, É. (Ekstern), Christensen, H. E. M. (Intern), Jancsó, A. (Ekstern)
Number of pages: 7
Pages: 90-96
Publication date: 2016
Main Research Area: Technical/natural sciences

Publication information

Journal: Protein Expression and Purification
Volume: 123
ISSN (Print): 1046-5928
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.667 SNIP 0.627 CiteScore 1.46
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.755 SNIP 0.79 CiteScore 1.55
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.815 SNIP 0.832 CiteScore 1.8
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.719 SNIP 0.719 CiteScore 1.61
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.716 SNIP 0.777 CiteScore 1.57
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.789 SNIP 0.787 CiteScore 1.66
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.796 SNIP 0.798
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.938 SNIP 0.827
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.11 SNIP 0.776

Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.025 SNIP 0.81
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 0.933 SNIP 0.731
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 0.852 SNIP 0.705
Scopus rating (2004): SJR 0.725 SNIP 0.701
Scopus rating (2003): SJR 0.875 SNIP 0.666
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 0.866 SNIP 0.586
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 0.914 SNIP 0.639
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 0.868 SNIP 0.689
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 0.99 SNIP 0.643
Original language: English
Metalloregulatory proteins, Cysteine, Copper(I), DNA binding, Four step purification
DOIs:
10.1016/j.pep.2016.03.012
Source: FindIt
Source-ID: 2303236428
Publication: Research - peer-review › Journal article – Annual report year: 2016